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Reduced Intensity Conditioning for Allogeneic Hematopoietic Cell Transplantation: Current Perspectives

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ABSTRACT

Allogeneic HCT after myeloablative conditioning is an effective therapy for patients with hematologic malignancies. In an attempt to extend this therapy to older patients or those with comorbidities, reduced intensity or truly nonmyeloablative regimens have been developed over the past decade. The principle underlying reduced intensity regimens is to provide some tumor kill with lessened regimen-related morbidity and mortality and then rely on graft-versus-tumor (GVT) effects to eradicate remaining malignant cells, whereas nonmyeloablative regimens rely primarily on GVT effects. In this article, 3 representative approaches are described, demonstrating the clinical application for hematopoietic and nonhematopoietic malignancies. Current challenges include controlling GVHD while allowing GVT to occur. In the future, clinical trials using reduced intensity and nonmyeloablative conditioning will be compared with myeloablative conditioning in selected malignancies to extend the application to standard-risk patients.

KEY WORDS

Reduced intensity • Nonmyeloablative • Allogeneic • Hematopoietic cell transplantation

INTRODUCTION

Allogeneic HCT was developed as a method to rescue patients from severe myelosuppression after traditional myeloablative chemoradiotherapy was given. HCT permitted intensification of the chemoradiotherapy well beyond the marrow-toxic range to the point at which nonhematopoietic toxicities became dose limiting. Although increasing doses of chemotherapy or radiation resulted in lower relapse rates, the survival was not improved due to higher nonrelapse mortality (NRM). In addition, the nonhematopoietic toxicities severely limited the application of allografting to patients <50 or 60 yr of age and who did not have significant comorbidities. Because the median age of patients with most candidate diseases ranges from 65 to 70 yr, most patients are not conventional transplantation candidates.

There are many factors responsible for disease control after HCT including disease burden at time of HCT, conditioning intensity, and graft source and composition. Considerable data indicate that much of the therapeutic benefit of allogeneic HCT is related to graft-versus-tumor (GVT) effects mediated by the donor-derived immunocompetent cells. The most direct evidence is the ability of DLIs to induce a remission in patients who have relapsed after HCT. The second line of clinical evidence comes from the observation of reduced risk of relapse in patients with GVHD. Other evidence includes increased risk of relapse in T cell-depleted or syngeneic HCT and the failure to eliminate minimal residual disease after T cell-replete HCT. Different reduced intensity and nonmyeloablative conditioning regimens have been explored for hematopoietic and nonhematopoietic malignancies. The conditioning regimens typically include a purine analog, such as fludarabine, an alkylating agent, or low-dose TBI. The principle underlying reduced intensity conditioning regimens is to provide tumor kill using drugs with proven activities against the targeted malignancies while waiting for GVT effects to occur. The cytotoxic conditioning also serves to suppress the host-versus-graft reactions. The second

approach of nonmyeloablative conditioning employs an immunosuppressive regimen that is minimally myelosuppressive. Postgrafting immunosuppression is used for the dual purposes of enhancing engraftment and controlling GVHD.

Over the past decade, many groups of investigators have explored variations of these less intensive preparative regimens. In this article, 3 representative approaches are described, demonstrating the clinical application for reduced intensity conditioning for hematopoietic and nonhematopoietic malignancies.

NONMYELOABLATIVE HCT USING LOW-DOSE TBI-BASED CONDITIONING REGIMENS

To extend the clinical use of allogeneic HCT to older patients and those with comorbidities, a truly nonmyeloablative HCT regimen was developed based on studies in a preclinical canine model [1]. Stable donor hematopoietic chimerism was achieved using 2-Gy TBI followed by postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF). This regimen was translated to patients in a multi-institutional clinical trial in recipients of HLA-identical sibling HCT using G-CSF mobilized PBSCs [2]. The hematologic changes were milder than usually observed after myeloablative or reduced intensity HCT, with most patients having full donor chimerism. Nonfatal graft rejections occurred in 20% of patients, primarily those who had not received preceding myelosuppressive chemotherapy, this was reduced to 3% with the addition of fludarabine (90 mg/m^2) to the 2-Gy TBI [3]. The same fludarabine and 2-Gy TBI regimen was used in recipients of 10/10 HLA-antigen matched unrelated donor grafts with use of extended MMF/cyclosporine immunosuppression [4]. Donor T cell chimerism and durable engraftment were higher in recipients of PBSCs compared to BM recipients, leading to exclusive use of PBSCs in subsequent trials using this nonmyeloablative regimen. Based on MMF pharmacokinetic studies, a subsequent trial of thrice daily, rather than twice daily, MMF successively reduced the risk of graft rejection in recipients of unrelated donor PBSC grafts [5].

Advantages of Reduced Intensity Conditioning Regimens

The most important benefit of a reduced intensity (including nonmyeloablative) conditioning regimen includes a reduction in morbidity and NRM, even in older patients and those with comorbidities. Only 47% of patients conditioned with the fludarabine/TBI regimen required hospitalization and the median duration of stay was 8 d [2]. There was a significant reduction in the need for RBC (63% versus 96%; $P = .001$) and platelet (23% versus 100%; $P < .0001$) transfusions in recipients of nonmyeloablative versus myeloablative conditioning [6]. Durations of neutropenia and early bacteremia were also reduced in the nonmyeloablative recipients, although there was no difference in the incidence of fungal disease [7]. The cumulative incidence of idiopathic pneumonia syndrome was significantly lower [8], and no case of veno-occlusive disease was observed [9].

Influence of Pretransplantation Comorbidities on Outcome

With the advent of reduced intensity and nonmyeloablative conditioning regimens, allogeneic HCT is currently being given to older and more heavily pretreated patients. In addition to the physiologic effects of aging, much of the increased morbidity and mortality is associated with pre-existing medical conditions related or unrelated to previous therapy for the underlying malignancy. The Charlson Comorbidity Index (CCI) was used to evaluate outcomes in patients undergoing nonmyeloablative and myeloablative conditioning before HCT [10,11]. The CCI was useful in predicting NRM and overall survival in recipients of related and unrelated donor grafts. The patients receiving nonmyeloablative conditioning had higher CCI scores and were older, yet had significantly lower NRM than did patients prepared with myeloablative regimens. More recently, an HCT-specific comorbidity index (HCT-CI) was developed that included comorbidities that were most relevant for HCT and was found to have a higher discriminative capacity [12]. The HCT-CI captured 62% of study patients with scores >0 compared with 12% with the CCI. Currently, the HCT-CI is being validated at multiple transplantation centers and, in the future, may be helpful in determining the most appropriate intensity of conditioning regimen for any given patient.

Chimerism and Graft Composition

The kinetics of donor engraftment were analyzed in patients with hematologic malignancies given related or unrelated donor grafts after 2-Gy TBI with or without fludarabine [13]. Although patients rapidly developed high degrees of donor engraftment, most remained mixed donor/host chimeras for up to 6 mo. Those given preceding chemotherapy and patients with PBSC grafts had the highest degrees of donor chimerism. Low donor T cell and NK cell chimerism levels on day 14 were associated with graft rejection, whereas high T cell chimerism on day 28 was associated with aGVHD. Earlier establishment of donor NK cell chimerism was associated with better progression-free survival. Achievement of full donor chimerism was associated with a decreased risk of progression or relapse [14]. These data suggest that monitoring peripheral blood subset chimerism might allow inter-

ventions soon after HCT to prevent rejection or relapse.

The effect of graft composition on chimerism was investigated in recipients of unrelated donor PBSC grafts. Higher numbers of CD34 cells were associated with higher levels of complete donor T cell chimerism at day +28 with a trend for less graft rejection [15]. Higher numbers of CD34 cells in related donor recipients had a beneficial effect on survival [16]. There were no associations between any cell subsets (including CD34) and aGVHD or cGVHD in the related or unrelated donor recipients.

GVHD and HCT Outcomes

In a retrospective analysis of patients 50-65 yr of age, the incidence of aGVHD was reduced in recipients of nonmyeloablative versus myeloablative conditioning, but no difference was observed in the cumulative incidence of cGVHD requiring therapy [17]. Nonmyeloablative HCT was associated with a syndrome of late onset aGVHD in some patients occurring beyond day 100, which may have relevance in developing regimens for GVHD prophylaxis.

Many studies had previously shown a close relation between GVHD and GVT responses after myeloablative HCT. GVT effects were investigated in 322 recipients of nonmyeloablative conditioning and related or unrelated donor grafts. Fifty-seven percent of patients with measurable disease achieved a complete (44%) or partial (13%) remission after HCT [14]. Grade II-IV aGVHD did not affect relapse/progression but was associated with increased NRM and poorer progression-free survival. Chronic GVHD was associated with decreased relapse/progression with better progression-free survival without increase in NRM. Currently, trials are ongoing to evaluate new approaches in GVHD prophylaxis to help improve survival.

Nonmyeloablative HCT after Failed High-dose HCT

Data were analyzed from 147 patients (median age, 46 yr) given an HLA-matched related (n = 62) or unrelated (n = 85) hematopoietic cell tranplant after failing a myeloablative autologous (n = 135), allogeneic (n = 10), or syngeneic (n = 2) HCT [18]. Three-year probabilities of NRM, progression-free, and overall survivals were 32%, 20%, and 27% in related and 28%, 28%, and 44% in unrelated recipients, respectively. Best outcomes were observed in patients with NHL, with 3-yr probabilities of progression-free survival of 57% and 56% in patients with mantle cell lymphoma and indolent NHL, respectively, whereas patients with Hodgkin lymphoma (HL) and multiple myeloma (MM) had higher incidences of relapse/progression, resulting in poorer outcomes.

Disease-specific Results

The following results in 3 different hematologic malignancies are from multi-institutional studies using fludarabine and 2-Gy TBI conditioning. Encouraging results were observed in 122 patients (related donor, n = 58; unrelated donor, n = 64) with AML [19]. Cumulative NRMs were 10% and 22% for related and unrelated donor recipients at 2 yr, respectively. The overall and progression-free 2-yr survivals were 48% and 44%, respectively. Patients undergoing transplantation in first CR from unrelated donors had higher 2-yr overall survival (63% versus 44%) and lower risk of relapse (16% versus 50%) than did recipients of related donor grafts, suggesting enhanced GVT effect mediated by unrelated donor cells.

Sixty-four patients with chemotherapy-refractory CLL were given HCT from HLA-matched related (n = 44) or unrelated (n = 20) donors after conditioning with fludarabine/2-Gy TBI [20]. Two-year estimates of NRM, progression-free, and overall survivals were 22%, 52%, and 60%, respectively. Unrelated HCT resulted in higher CR and lower relapse rates than did related HCT.

HLA-matched related (n = 16) and unrelated (n = 17) donor HCTs were carried out in patients with relapsed or refractory mantle cell lymphoma including 14 patients whose previous autologous HCT had failed [21]. The 2-yr probabilities of NRM, progression-free, and overall survival were 24%, 60%, and 65%, respectively. Results suggest that mantle cell lymphoma is responsive to GVT effects.

Future Directions

Nonmyeloablative conditioning using fludarabine/2-Gy TBI provides a potentially curative option for patients who were previously considered ineligible for HCT. Patients have consistent engraftment and evidence exists for GVT effects. We are currently carrying out a prospective, phase III, randomized trial evaluating nonmyeloablative versus myeloablative HCT for patients with myelodysplastic syndrome or AML in CR at the time of HCT. Other research is directed at decreasing GVHD incidence and thus minimizing the need for prolonged steroid use (which puts patients at risk for infectious complications) with subsequent reduction of NRM. To expand the donor pool, ongoing trials are using HLA-mismatched, including haploidentical, donors. For patients with aggressive diseases, studies are investigating the combination of nonmyeloablative conditioning with "disease-targeted" therapy such as radiolabeled mAbs, imatinib, or rituximab. Investigation regarding tumor antigens and tissue-specific mHAs may allow administration of tumor-specific cytotoxic T cells to allow for more GVT effects without increasing the risk of GVHD.

ALEMTUZUMAB AND REDUCED INTENSITY TRANSPLANTATION FOR LYMPHOMA

The Antibody and Pharmacokinetics

Alemtuzumab (CAMPATH-1H) is a humanized IgG1 monoclonal antibody directed against the CD52 antigen, which is widely expressed on all human lymphoid cells except terminally differentiated plasma cells [22]. Delayed clearance of the mAb may impair immune reconstitution, affect rates of viral reactivation, and limit efficacy of the donor T cell-mediated GVT effect derived from the graft itself, early adoptive immunotherapy, or later DLI. By administering the Ab to the recipient as part of the conditioning regimen, it will result in T cell, B cell, and selected DC depletion of the recipient [23]. Among myeloid DCs, CD52 expression is limited to circulating and monocyte-derived DCs [24]. Neither Langerhans cells nor dermal-interstitial DCs express CD52, whether resident in tissues or generated ex vivo with cytokines [24]. In addition, if sufficient Ab is circulating on the day of transplantation, this will result in T cell depletion of the graft, thereby potentially reducing the incidence and severity of GVHD. The half-life of alemtuzumab in humans is dependent on the amount of target CD52 antigen in the patient. After a dose of 20 mg/d for 5 d (days −8 to −4) in vivo before allogeneic HCT, there is persistence of alemtuzumab in vivo past day 0 sufficient to cause T cell lysis by complement fixation and antibody dependent cell mediated cytotoxicity (ADCC) [25]. Using this dose schedule, significant levels of Ab persist through day 28 after transplantation. The optimal dose of antibody to prevent GVHD and minimize post-transplantation immune suppression is currently unknown.

Reduced intensity Regimens Incorporating Alemtuzumab

The most commonly used regimen combines alemtuzumab with fludarabine and an alkylating agent, usually melphalan or busulfan [26,27]. Alemtuzumab has also been added to the carmustine/etoposide/cytarabine/melphalan (BEAM) regimen and used in reduced intensity conditioning for lymphoma [28]. These regimens vary in their myeloablative and immunosuppressive properties, and it is currently not known which regimen is optimal for any given clinical scenario.

Toxicity

Reduced intensity conditioning regimens that include alemtuzumab have been generally well tolerated. Although many of the regimens containing melphalan or busulfan will result in 5-7 d of neutropenia, avoidance of methotrexate for GVHD prophylaxis minimizes mucositis and allows most patients to continue to eat relatively normally. However, there is infusional toxicity related to alemtuzumab. This is secondary to a cytokine release syndrome that can cause fever, skin rashes, hypotension, and occasionally bronchospasm. This can be reduced by using a premed of methylprednisolone 2 mg/kg i.v. administered before the first 20-mg dose of alemtuzumab. This is very effective at preventing the cytokine release. Occasional patients may require corticosteroids after the first day of infusion.

Engraftment and Chimerism

The largest experience has used alemtuzumab in combination with fludarabine and melphalan. Using this regimen, the median time to recover an absolute neutrophil count of 0.5×10^9/L was 13 d (8-23 d) and a count $>1.0 \times 10^9$/L was 17 d (8-47 d). The median time to achieve a platelet count $>20 \times 10^9$/L was 13 d (range, 3-96 d) and a count $>50 \times 10^9$/L was 17 d (8-118 d). Incidences of graft rejection were <3% using PBSCs from sibling donors and 6% with BM from unrelated donors [29]. Lineage-specific chimerism studies have been performed using microsatellite PCR in patients after this regimen. Three patterns of chimerism have been documented using this regimen:

1. Fully donor in all lineages
2. Mixed chimera in all lineages
3. Fully donor myeloid chimerism with mixed T cell chimerism

Because reduced intensity transplantation is heavily dependent on GVT effects, the development of mixed T cell chimerism (a marker of bidirectional immune tolerance) could be associated with a higher incidence of disease relapse. Therefore, if mixed chimerism persists once immunosuppression has been discontinued, attempts to promote full donor chimerism and graft-versus-leukemic activity using DLI might reduce disease recurrence. Most patients with stable mixed chimerism will achieve full donor chimerism after administration of DLIs (see below).

Graft-versus-Host Disease

Perhaps the most impressive effect of alemtuzumab as part of a reduced intensity conditioning regimen is in the prevention of GVHD. Published results of sibling donor HCT using other nonmyeloablative conditioning regimens have shown a 38%-60% incidence of grade II-IV aGVHD that is the primary cause of death in some patients. However, when alemtuzumab has been used as part of the conditioning regimen using HLA-identical siblings, most patients do not develop any GVHD, and the reported incidence of grade II-IV aGVHD after HLA-identical sibling transplantation was 5% [26]. When transplants using unrelated donors are assessed, the effects of alemtuzumab in not only preventing GVHD but also

limiting TRM are particularly impressive. For reduced intensity regimens that do not include alemtuzumab, the reported experience of unrelated donor HCT using a fludarabine-plus-melphalan protocol observed high rates of severe GVHD, with 1 in 4 patients dying directly as a result of GVHD. In contrast, a similar regimen containing alemtuzumab was associated with a low incidence of GVHD despite significant HLA disparity in many transplants. Only 6% of patients had grade III-IV aGVHD, and only 15% developed grade II aGVHD [29].

Hodgkin Lymphoma

The therapeutic options for patients with HL who relapse soon after completion of first-line chemotherapy or whose autologous transplant has failed are very limited. The efficacy of an allogeneic myeloablative transplant in HL remains controversial, and possible benefit was eliminated by very high NRM using TBI-based conditioning regimens. An alemtuzumab-based reduced intensity conditioning regimen has been reported in 49 patients with HL [30]. Median age was 32 yr; number of prior treatment lines was 5; and time from diagnosis was 4.8 yr. Forty-four patients had undergone prior autologous transplantation. Thirty-one had HLA-matched related and 18 HLA-unrelated donors. Median follow-up was 967 d. Grade II-IV aGVHD occurred in 12% before DLI and cGVHD in 16%. Sixteen received DLI beginning 3 mo after transplantation for residual disease/progression. Six developed grade II-IV aGVHD and 5 developed cGVHD. Nine demonstrated disease responses after DLI (7 complete, 2 partial). NRM was 15% with an additional 9% mortality associated with DLI (overall procedural mortality, 24%; 17% related versus 39% unrelated donors; $P = .06$). Projected 4-yr overall and current progression-free survivals were 59% and 39%, respectively (66% and 41% for related donors). These data strongly support a clinically relevant graft-versus-HL effect and demonstrate the potential for durable responses even in this group of heavily pretreated HL patients. The relatively low procedural mortality allows consideration of application sooner in the disease course, particularly in the related donor cohort, and support further exploration of allogeneic therapies.

Non-Hodgkin Lymphoma

Results in 121 patients with NHL/CLL who underwent transplantation using an alemtuzumab, fludarabine, and melphalan regimen were recently reported [31]. Diagnoses were in 3 categories: low-grade follicular NHL (n = 50), mantle cell lymphoma (n = 21), and high-grade NHL (n = 50, including transformed low-grade disease in 15). Donors were HLA-matched siblings in 75 (62%) and unrelated in 46 (38%), 18 of whom were HLA-mismatched at up to 3/10 loci. Forty-eight percent of patients had failed previous autologous HCT. Median follow-up was 35 mo (1-78 mo). For the group with low-grade follicular NHL (n = 50), estimated overall survivals were 76% at 1 yr and 67% at 4 yr, and NRM was 15% at 4 yr. Disease relapse or progression occurred in 12 patients, of whom 8 received DLI, with responses in 6. Current progression-free survival is 69% at 4 yr (Figure 1A). For the group with mantle cell lymphoma (n = 21), estimated overall survival was 83% at 4 yr, NRM was 11% at 4 yr, and relapse or progression occurred in 6 patients. Three patients received DLI, with nonsustained responses in 2. Current progression-free survival is 43% at 4 yr. For high-grade NHL (n = 50), estimated overall survivals were 52% at 1 yr and 45% at 4 yr. Prior autologous HCT was common in this group (72%), and NRM was higher at 34% at 1 yr and 37% at 4 yr. Progression/relapse occurred in 15 patients, of whom 10 received DLIs, with responses in 5. Current progression-free survivals are 48% at 1 yr and 38% at 4 yr (Figure 1B).

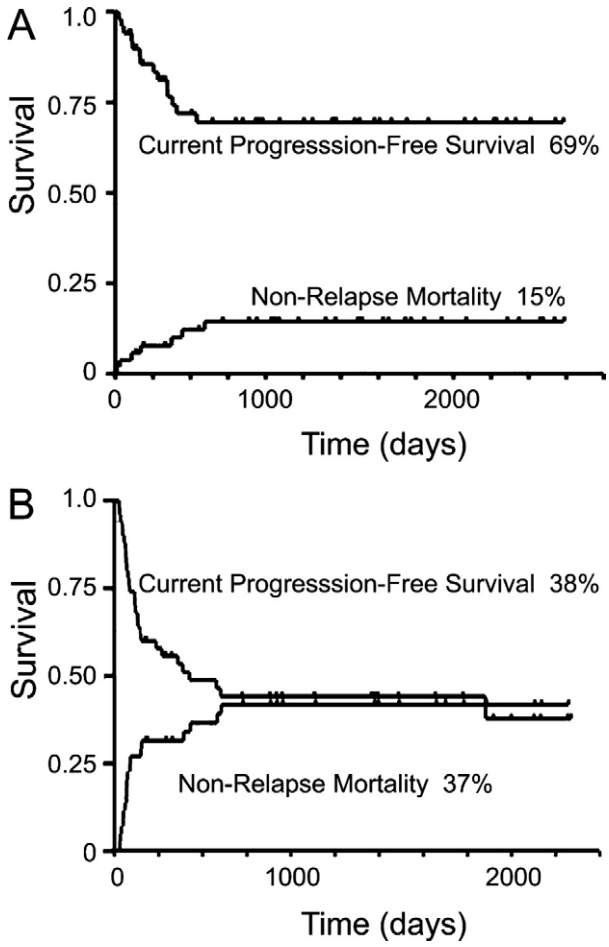

Figure 1. NRM and current progression-free survival for (A) follicular lymphoma and (B) aggressive lymphoma.

Donor Lymphocyte Infusion

After reduced intensity conditioning, the presence of mixed chimerism or residual tumor are risk factors for disease recurrence. DLI can promote full donor chimerism and graft-versus-leukemic effects but are associated with a high risk of GVHD early after transplantation. Although DLI with a T cell content of 1×10^7/kg is relatively safe if administered >1 yr after transplantation [32], there are few data on the effective and safe dose of DLIs that can be given early after transplantation. A trial of DLI after a reduced intensity alemtuzumab-containing regimen has been reported [33]. The number of patients with NHL given this treatment has increased since the time of that publication. Sixty-eight infusions of dose-escalating DLI have been administered to 28 allogeneic transplant recipients. The diagnoses were indolent NHL (n = 23) and transformed NHL (n = 5). Indications for DLI were progressive disease with/without mixed chimerism (n = 17) or persistent mixed chimerism alone (n = 11). Escalating doses of cells were administered in the absence of GVHD for persistent mixed chimerism or until disease response. Thirteen of 16 evaluable patients (81%) treated for disease progression showed a significant response to DLI, and all 23 evaluable patients treated for mixed chimerism converted to stable donor hematopoiesis (100%). The major toxicity resulting from the use of DLI was GVHD. The cumulative incidence of grade II-IV aGVHD was 15%, and that of extensive cGVHD was 35%; there were no deaths due to GVHD. Seven patients had graft-versus-lymphoma responses without significant GVHD. These data support the existence of a clinically significant GVT effect in NHL and indicate that this is an effective treatment for progressive disease after allogeneic HCT.

Summary

Alemtuzumab reduces the incidence of aGVHD and cGVHD after reduced intensity conditioning HCT and reduces GVHD-related mortality. There is a delay in immune reconstitution and an increased incidence of viral infections with the use of alemtuzumab; however, in the case of CMV, this does not adversely affect TRM. Many patients develop mixed chimerism with this approach and full donor chimerism can usually be achieved after DLI. In patients with indolent lymphoma and HL, these DLIs are associated with impressive graft-versus-lymphoma responses. The optimal dose of alemtuzumab remains unknown. Whether this antibody will improve long-term progression-free survival compared with other reduced intensity conditioning regimens using conventional GVHD prophylaxis also remains unknown.

ALLOGENEIC TRANSPLANTATION FOR NONHEMATOLOGIC CANCERS

Allogeneic T cells are capable of generating antileukemic effects in a number of hematologic malignancies after HCT. This GVT effect is powerful enough to cure patients with hematologic cancers who have become completely resistant to chemotherapy. Over the past 20 yr, the list of malignant diseases shown to be susceptible to the GVT effect has grown to include acute and chronic leukemias, post-transplantation EBV-associated lymphoproliferative disorder, HL, NHL, and MM.

The failure of conventional chemotherapy to improve survival in many metastatic cancers has provided an impetus for the development of immune-based treatment approaches for solid tumors. Initial efforts were directed at nonspecific enhancement of innate immunity through the administration of immunomodulatory cytokines. Unfortunately, cytokine-based immunotherapy has proved to be inactive in most solid tumors. Perhaps the greatest limitation of conventional immunotherapy regimens is that they attempt to enhance a host immune system rendered dysfunctional by prior chemotherapy treatment or longstanding exposure to the immunosuppressive effects of the tumor. Allogeneic HCT, a procedure that culminates in complete immune replacement, could potentially overcome this problem and at the same time expand the repertoire of immune cells capable of recognizing tumor antigens, including tumor-reactive T cells that recognize polymorphic variants of mHAs. The introduction of reduced intensity conditioning regimens that have decreased TRM and preserved GVT effects has recently led investigators to explore the potential of allogeneic immunotherapy to treat incurable metastatic cancers [34,35].

Since 1999, there have been a number of publications describing GVT effects in patients with a variety of solid tumors undergoing reduced intensity HCT [36,37]. At present, 12 case series describing the results of nonmyeloablative HCT for renal cell carcinoma (RCC) and 5 small series of HCT in breast cancer have been reported. Although partial responses have most often been observed in patients having evidence for a GVT effect, a subset of patients had dramatic complete responses that have proved to be durable. Numerous case reports and a few case series reporting evidence for GVT effects in other solid tumors including metastatic pancreatic carcinoma, colon carcinoma, ovarian carcinoma, and sarcomas have also been reported over the past 2 yr [34]. In several

series, survival has been reported to be significantly superior in patients having a GVT effect compared with nonresponders [38-40].

Allogeneic HCT for Metastatic RCC

Since 1998, we and others have conducted pilot trials investigating for GVT effects after nonmyeloablative allogeneic HCT in patients with metastatic RCC. Twelve studies evaluating a variety of nonmyeloablative allogeneic transplantation approaches for metastatic RCC have thus far been reported (Table 1) [38-50]. In 9 of these series, disease regression compatible with a GVT effect has been observed to occur in a subset of patients. Although most of these series have been small, they have demonstrated the feasibility of nonmyeloablative HCT for metastatic RCC and illustrated the following principles:

- Conversion from mixed to full donor chimerism can be accelerated by withdrawal of immunosuppression and/or DLI and is important to enhance a GVT effect.
- Acute GVHD correlates with donor immune-mediated tumor responses.
- RCC resistant to cytokine therapy, including IFN-α and high-dose IL-2, may respond after nonmyeloablative HCT.
- Development of a GVT effect is delayed after HCT, underscoring the importance of careful selection of patients with sufficient pretransplantation life expectancy.
- Response rates have been extremely variable, with a range of 8%-57%. The familiarity of transplantation centers with this approach and protocol-specific selection criteria used to determine patient eligibility have contributed to this variability.

The largest experience to date of allogeneic HCT for metastatic RCC was recently reported from a pooled series of 21 different European transplantation centers [38]. One hundred twenty-four patients underwent transplantation using a variety of different transplantation regimens. A tumor response was observed in 28 of 98 evaluable patients (cumulative incidence, 32%), including 24 partial responses and 4 complete responses. In a multivariate analysis, tumor responses were associated with aGVHD, use of an HLA-mismatched donor, and a period <1 yr from diagnosis of metastatic disease to transplantation. In a multivariate analysis of overall survival, the presence of cGVHD, DLI after transplantation, and having <3 metastatic sites were associated with improved survival after HCT.

Experience in humans has shown that allogeneic GVT effects are most effective when performed during a state of minimal residual disease. The intrinsic delay to tumor regression makes careful selection of patients for the procedure obligatory. A recent report has shown that performance status, C-reactive protein levels, and lactate dehydrogenase levels could be used in a Cox regression model to predict survival after allogeneic HCT, potentially providing a tool to assist clinicians in selecting patients who would be appropriate candidates for this approach [51].

The identification of mechanisms through which graft-versus-RCC effects occur and the antigens expressed on RCC cells that serve as targets for donor immune cells remain active areas of investigation [52,53]. Recently, T cells with in vitro tumor cytotoxicity patterns consistent with recognition of mHAs and tumor-restricted antigens have been identified in some responding patients [45,54]. The identification of tumor-restricted antigens targeted by donor immune cells could lead to the development of transplantation approaches that enhance GVT effects and avoid GVHD by incorporating strategies such as tumor vaccination or the adoptive infusion of in vitro expanded donor T cells with tumor-antigen specificity.

Table 1. *Published Series of Nonmyeloablative HCT to Treat Metastatic RCC*

Study	n	Conditioning	aGVHD, %	cGVHD, %	TRM, %	Response Rate, %
Childs et al [37]	19	Flu + Cy	53	21	11	53
Rini et al [41], Artz et al [50]	18	Flu + Cy	22	39	14	22
Bregni et al [42]	7	Flu + TT	86	71	0	57
Pedrazzoli et al [43]	7	Flu + Cy	0	N/A	29	0
Blaise et al [44]	25	Flu + Bu + ATG	42	60	9	8
Nakagawa et al [46]	9	Flu/Cla + Bu + ATG	44	44	0	11
Ueno et al [40]	15	Flu + Mel	47	27	33	20
Hentschke et al [47]	10	Flu + TBI ± ATG	50	30	40	0*
Massenkeil et al [48]	7	Flu + Cy + ATG	29	57	14	29
Tykodi et al [45]	8	Flu + TBI	50	50	13	13
Rini et al [49]	22	Flu + Cy	50	23	9	0
Barkholt et al [38]	124	Multiple variables	40	33	16	32

Bu indicates busulfan; Cla, cladribine; Cy, cyclophosphamide; Flu, fludarabine; Mel, melphalan; N/A, not available; TBI, 200-cGy TBI; TT, thiotepa.

*Mixed responses observed.

We have successfully generated donor $CD8^+$ T cell clones from lymphocytes obtained from responding patients that have direct cytotoxicity against the patient's RCC cells. Using cDNA expression cloning, we recently identified a tumor antigen recognized by an HLA-A 11 restricted RCC-specific T cell clone isolated from a patient at the time of tumor regression (donor in origin). Importantly, this antigen was found to be derived from a newly discovered gene expressed in >50% of RCC lines but not in any normal tissues (Takahashi Y, Harashima N. et al, 2006, unpublished observations).

Allogeneic HCT for Breast Cancer

Recent reports of GVT effects occurring in women with metastatic breast cancer after reduced intensity conditioning regimens have provided a renewed impetus to study HCT as immunotherapy for this disease (Table 2) [40,42,44,55,56]. As observed in patients with RCC having a GVT effect, tumor regression has most often been described to occur after full donor chimerism is achieved, often in association with GVHD or after DLI.

An innovative approach using tandem autologous followed by allogeneic HCT for patients with heavily pretreated metastatic breast cancer was recently reported by investigators from Italy [56]; in 3 of 17 patients, durable complete responses ongoing >1320, 1530, and 2160 d after HCT were observed. Together these results establish the existence of GVT effects against metastatic carcinoma of the breast, which in select patients may lead to considerable DFS. Further prospective studies are clearly warranted to better evaluate the potential of allogeneic HCT for this solid tumor type.

Future Directions

Nonmyeloablative transplantation trials showing GVT effects in metastatic solid tumors provide further proof of the strength of allogeneic immunotherapy. Trials evaluating nonmyeloablative approaches using unrelated donors for patients with metastatic RCC have recently been initiated and, if effective, could potentially expand the application of allogeneic immunotherapy to a far larger number of patients. However, the use of allogeneic HCT to treat solid tumors will likely remain limited until the safety and efficacy of the approach are improved. Concerns over transplant-associated complications continue to make referring oncologists reluctant to send patients for these investigational studies. As a consequence, the approach is further limited by its obligate enrollment of patients with advanced, treatment refractory disease, often with life expectancies too short to benefit from a delayed GVT effect [36]. Further progress in the field will require the development of strategies that limit GVHD and target the allogeneic T cells to the tumor. The development of novel regimens that incorporate maneuvers to control or shrink the tumor to buy time for a GVT effect to occur could also be used to improve transplantation outcome. The feasibility of incorporating receptor tyrosine kinase inhibitors including sunitinib and sorafenib and the vascular endothelial growth factor antibody bevacizumab after allogeneic HCT for RCC is currently being evaluated at the National Heart, Lung, and Blood Institute. Another strategy to enhance GVT effects against RCC is the use of alloreactive KIR-incompatible NK cells in the transplantation regimen. In vitro, allogeneic KIR-incompatible NK cells have enhanced cytotoxicity against RCC cells compared with autologous or KIR-matched NK cells [57]. Adoptive infusion of alloreactive donor NK cells was recently found to significantly reduce the risk of aGVHD and potentiate graft-versus-RCC effects in a murine model of HCT for kidney cancer (Ludqvist A, et al, unpublished observations). Trials evaluating adoptively infused allogeneic NK cells and other novel methods to direct/target alloimmune cells against the cancer in humans undergoing HCT for metastatic RCC will likely be explored in the near future.

CONCLUSIONS

Results of reduced intensity and nonmyeloablative HCT studies over the past decade are encouraging, and the knowledge gained thus far is instrumental in the design of clinical trials. Less toxic regimens can be implemented and preserve potent GVT effects in hematologic and nonhematologic malignancies. Current challenges include optimizing the regimen to still allow for a GVT effect while minimizing toxicities,

Table 2. *Published Series of Nonmyeloablative HCT to Treat Metastatic Breast Cancer*

Study	n	Conditioning	aGVHD (%)	cGVHD (%)	NRM, %	Response Rate (%)
Ueno et al [40]	8	Flu + Mel	2 (25)	6 (75)	0	2 CR (25)
Bregni et al [42]	6	Flu + TT	2 (33)	2 (33)	0	2 PR (33)
Blaise et al [44]	12	Flu + Bu + ATG	5 (42)	7 (60)	9	1 CR, 1 PR (17)
Bishop et al [55]	16	Flu + Cy	9 (56)	4 (25)	13	2 PR (13)
Carella et al [56]	17	Flu + Cy	5 (29)	6 (35)	24	3 CR, 1 PR (24)

Bu indicates busulfan; Cy, cyclophosphamide; Flu, fludarabine; Mel, melphalan; PR, partial remission; TT, thiotepa.

including serious GVHD and infections. Further progress in adoptive transfer of T or NK cell populations with relative tumor specificity in combination with disease-targeted therapy would make reduced intensity HCT even more effective. Prospective disease-specific randomized trials evaluating reduced intensity or nonmyeloablative regimens versus myeloablative regimens in younger patients are needed to define the role in this population for reduced intensity HCT.

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